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THE BACTERICIDAL AND PROTOZOACIDAL ACTIVITY OF EMETIN HYDROCHLORID IN VITRO*

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While there are in the medical and dental professions differences of opinion regarding the rôle of Endamoeba gingivalis, Gros, in the etiology of pyorrhea alveolaris and the efficacy of emetin in the treatment of this disease, as originally discovered and announced by Smith and Barrett[1] and independently by Bass and Johns,[2] and confirmed by numerous investigations by Smith, Middleton, and Barrett,[3] Evans and Middleton,[4] Chaplin,[5] and others, experimental investigations, especially those of Vedder[6] and Wherry,[7] have shown conclusively the high amebacidal action in vitro of ipecac and its chief alkaloid emetin.

Investigation of the probable bactericidal action of ipecac and of the alkaloid emetin has been incomplete. It is well known that various bacteria, including streptococci, pneumococci, various bacilli, spirochetes, leptothrices, etc., may be found in abundance in pus from the gums of persons suffering with pyorrhea alveolaris, and most observers, including those who advocate emetin in the treatment of this disease, assign to these bacterial species some rôle, usually a collateral one, in the etiology of this disease. Smith and Barrett[8] incline to the belief that the ameba act "in symbiotic relation with some or all of the vegetable organisms with which they are in association in nature; that by their proteolytic power they prepare a highly fitting pabulum for the growth of bacteria in the form of end products of their digestion of leukocytes, red blood cells and perhaps fixed cells as well, and thus favor a rank mycotic growth about them; that by their ingestion and destruction of these bacteria they set free a not inconsiderable amount of bacterial toxins of different kinds and of varying influences; and that these toxins are locally necrosing and of essential importance in determining and maintaining the gingival and alveolar inflammation, and too may be diffused and be productive of a widespread series of complications in the body of the host, commonly discussed in connection with the 'oral sepsis' of Hunter." Bass and Johns,[2] taking a more decided view of the rôle of the amebae in the pathogenesis of Rigg's disease, regard them as the specific cause.

* Received for publication September 21, 1915.

[1] Dental Cosmos, 1914, 56, p. 948.

[2] New Orleans Med. and Surg. Jour., 1914, 68, p. 456. Jour. Am. Med. Assn., 1915, 64, p. 553.

[3] Jour. Am. Med. Assn., 1914, 63, p. 1746.

[4] Ibid., 1915, 64, p. 422.

[5] Dental Cosmos, 1915, 57, p. 189.

[6] Jour. Am. Med. Assn., 1914, 62, p. 501. Bull. Manila Med. Soc., 1911.

[7] Jour. Infect. Dis., 1912, 10, p. 162.

[8] Oral Health, 1915, 5, p. 137.

In view of the fact that the associated micro-organisms disappear from the pockets of disease with the cessation of suppuration, tho not as completely as do the amebae, and that temporary improvement of the lesions is frequently obtained and occasionally a permanent cure effected by cleansing and treatment with various antiseptics and by bacterial vaccine therapy, we have investigated in more detail the probable bactericidal properties of emetin, with the hope of shedding more light on the action of this drug in the treatment of pyorrhea alveolaris, either by showing that the drug has a combined amebacidal and bactericidal action, or, in case the latter were absent or so feeble as to be without commensurate influence, to add thereby to the evidence indicating the intimate rôle of amebae in the pathogenesis of pyorrhea alveolaris and the efficiency of emetin in its treatment.

SCOPE OF THE INVESTIGATION

We have studied the bactericidal action of emetin hydrochlorid in vitro, in fluid and solid culture media, on the following micro-organisms: Staphylococcus aureus, Streptococcus salivarius, B. typhosus, B. anthracis, and B. subtilis. These bacteria were selected as test organisms mainly because their hardy character and their ability to grow uniformly in ordinary culture media make them adaptable for germicidal studies. The bacillus of anthrax was included because of the time-honored and empirical custom in the Philadelphia Hospital for Contagious Diseases of treating the wound of anthrax, after excision of the lesion, with powdered ipecac; one of us (Kolmer) has observed that in a number of cases so treated the drug may have been responsible for the destruction of anthrax bacilli in the wound. As will be shown later in this report, emetin hydrochlorid was found in our experiments to possess a relatively high bactericidal power for the spore-bearing anthrax bacillus, and in order further to study this action Bacillus subtilis was included in the list of test organisms. We have included also a study of the trypanocidal action of this alkaloid in vitro on Trypanosoma lewisi and Trypanosoma equiperdum, as well as on Endamoeba gingivalis, Gros.

In view of the results of this study, showing that emetin hydrochlorid possesses some bactericidal action, and in view of the beneficial results reported by Bass and Johns and others in the treatment of pyorrhea alveolaris and various complications, especially arthritic conditions (Evans and Middleton), with the hypodermatic administra-

tion of emetin, we have also studied the bactericidal action of emetin in vivo, the results of this study being given in a separate communication.

In the studies here reported, we have used dilutions of pure phenol as control germicide, so that the bactericidal value of emetin in the various methods employed and with the different test micro-organisms is expressed in terms of comparison with phenol.

BACTERICIDAL ACTION OF EMETIN HYDROCHLORID

In 1910 Vedder[9] reported that ipecac possessed no specific bactericidal effect against B. dysenteriae, notwithstanding that his studies clearly indicate the antiseptic, or even bactericidal, value—tho of undetermined degree—of ipecac in the case of the Shiga and Flexner strains of B. dysenteriae, B. typhosus and B. paratyphosus, and Staphylococcus aureus. Hitchens[10] found that a 4% solution of emetin failed to kill B. typhosus in 15 minutes. Frazier[11] found that a dilution of 1 : 25,000 in serum agar possessed antiseptic and even germicidal power for B. typhosus. Wherry[7] found emetin in solution of 1 : 20,000 bactericidal in 48 hours against the symbiotic bacillus in the ameba culture with which he worked.

In all our work tablets of emetin hydrochlorid (⅓ grain = 0.0215 gm.) for hypodermatic use, were used. Various dilutions (4, 2, 1, 0.5, and 0.25% solutions) in plain sterile neutral broth were freshly prepared for each experiment.

In all experiments the test micro-organism was grown for 24 hours in plain neutral broth and filtered through sterile filter paper before being used. Streptococcus salivarius was cultivated in serum broth. The cultures of B. anthrax and B. subtilis were briefly shaken with glass beads before filtration in order to break up chains of bacilli. In all instances the filtered cultures were slightly less dense than before filtration, but filtration was considered necessary in order to remove clumps of bacteria; it in no way interfered with the experiments.

In all experiments corresponding dilutions of pure phenol in sterile distilled water were employed. Likewise, each experiment had several culture controls to assure us that the culture was viable, able to multiply in the media used, and that the dose was sufficient.

With Rideal-Walker and Hygienic Laboratory Methods.[12]—Several experiments were conducted according to these methods with dilutions of emetin controlled by dilutions of phenol.

Table 1 is representative of the results obtained. In this experiment the temperature of medication was 20 C. A filtered 24-hour extract-

[9] Bull. Manila Med. Soc., 1911.
[10] Personal communication.
[11] Med. Rec., 1915, 87, p. 476.
[12] Jour. Infect. Dis., 1911, 8, p. 1.

broth culture of B. typhosus was used, the proportion of culture to disinfectant being 0.1 c.c. to 5 c.c. Extract broth was employed as subculture medium, reaction neutral to phenolphthalein. Results were read after 24 hours.

TABLE 1

RESULTS OF AN EXPERIMENT SHOWING BACTERICIDAL ACTION OF EMETIN AGAINST B. TYPHOSUS WITH RIDEAL-WALKER AND HYGIENIC LABORATORY METHODS

Sample	Dilution	Results Over a Period of 15 Minutes					
		2½	5	7½	10	12½	15
Phenol	1:20	—	—	—	—	—	—
Phenol	1:30	—	—	—	—	—	—
Phenol	1:40	—	—	—	—	—	—
Phenol	1:50	+	+	—	—	—	—
Phenol	1:80	+	+	+	+	—	—
Phenol	1:100	+	+	+	+	+	+
Emetin	1:20	+	+	+	+	+	+
Emetin	1:30	+	+	+	+	+	+
Emetin	1:40	+	+	+	+	+	+
Emetin	1:50	+	+	+	+	+	+
Emetin	1:60	+	+	+	+	+	+
Emetin	1:100	+	+	+	+	+	+

Both methods yielded similar results. It is to be noted that a 1:20 solution of emetin failed to kill B. typhosus in the longest interval of exposure—15 minutes; whereas a 1:80 dilution of phenol proved germicidal in this time. In this respect our results confirm those of others, but further experiments have shown that emetin possesses antiseptic and germicidal properties when the time of exposure is longer continued. Nevertheless, it should be added here that Barrett and Campbell (personal statement) have found that B. typhosus was not killed by exposure to as low dilution as 1:200 of emetin hydrochlorid in neutral broth in 96 hours. The only difference apprehended was that these investigators did not use a filtered culture for inoculation and employed a different strain from that used in our experiments. Possibly clumping may have prevented proper penetration of the culture.

With the Test-Tube Method.—Further experiments were conducted with the test-tube method as used by Schamberg and Kolmer[13] for testing the germicidal activity of substances insoluble in water.

Solutions of emetin—4, 2, 1, 0.5, and 0.25%—were prepared in plain, sterile, neutral broth, and similar dilutions of pure phenol in distilled water. With a sterile 1-c.c. volumetric pipet definite amounts of a given dilution of germicide were placed in a series of 6 test tubes (plugged with cotton and sterilized beforehand) as follows: 0.1, 0.2, 0.4, 0.6, 0.8, and 1 c.c. Sterile broth was then added to each tube with a sterile 5- or 10-c.c. volumetric pipet until the total

[13] Jour. Am. Med. Assn., 1914, 62, p. 1950.

quantity in each tube was brought up to 4.9 or 9.9 c.c. To each tube and a control tube of plain broth without germicide, was added 0.1 c.c. of a 24-hour filtered culture of the test micro-organism. The tubes then contained 5 or 10 c.c., according to the dilution desired; usually we worked with 5 c.c.

Tables 2 and 3 show the dilutions of germicide, secured in this manner, that act on the bacteria added to the tubes.

TABLE 2

DILUTIONS OF GERMICIDAL SUBSTANCE OBTAINED WHEN THE TOTAL AMOUNT IS MADE 5 C.C.

Stock Dilutions	Final Dilution When Diluent is Added to 5 c.c.					
	0.1 c.c.	0.2 c.c.	0.4 c.c.	0.6 c.c.	0.8 c.c.	1.0 c.c.
4%	1:1250	1:625	1:312	1:208	1:156	1:125
3%	1:1666	1:833	1:416	1:277	1:208	1:166
2%	1:2500	1:1250	1:625	1:416	1:312	1:250
1%	1:5000	1:2500	1:1250	1:833	1:625	1:500
0.5%	1:10,000	1:5000	1:2500	1:1666	1:1250	1:1000
0.25%	1:20,000	1:10,000	1:5000	1:3333	1:3500	1:2000

TABLE 3

DILUTIONS OF GERMICIDAL SUBSTANCE OBTAINED WHEN THE TOTAL AMOUNT IS MADE 10 C.C.

Stock Dilutions	Final Dilution When Diluent is Added to 10 c.c.					
	0.1 c.c.	0.2 c.c.	0.4 c.c.	0.6 c.c.	0.8 c.c.	1.0 c.c.
4%	1:2500	1:1250	1:624	1:416	1:312	1:250
3%	1:3333	1:1666	1:833	1:277	1:416	1:333
2%	1:5000	1:2500	1:1250	1:832	1:624	1:500
1%	1:10,000	1:5000	1:2500	1:1666	1:1250	1:1000
0.5%	1:20,000	1:10,000	1:5000	1:3332	1:2500	1:2000
0.25%	1:40,000	1:20,000	1:10,000	1:6666	1:5000	1:4000

Higher dilutions than these may be obtained by using higher stock dilutions; finer gradations may be secured by using 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 c.c. of stock dilution.

The tubes were then incubated at 37 C., and the results read and recorded each day over a period of 8 to 10 days. The controls were first inspected; they showed a good growth after the first 24 hours. As a rule, it was sufficient to inspect the tubes to learn the results: when the broth was perfectly clear, the result was set down for that day as negative or "germicidal"; if cloudy, the result was positive, the dilution of substance having failed to kill the test micro-organism.

By cultivating the tubes over a period of 10 days it was possible to determine the germicidal values of the substance tested. During the first 4 or 5 days the bacteria grew up in successive dilutions, thereby showing that their multiplication had been hindered through an antiseptic action of the substance but not killed. Very occasionally the bacteria in a tube grew up after the 7th day, but in no instance did we observe this occurring after the 8th or the 10th day. After this time all clear tubes were subcultured on slants of plain or glucose neutral agar to test their sterility; at the same time a number of those in which bacteria had grown up were subcultured to determine whether or not the micro-organisms had undergone spontaneous death. These subcultures were made by transferring several 4-mm. loopfuls of broth to the

slant of agar. With the micro-organisms used by us the broth cultures (i. e. the controls) were found viable at the end of 10 days; in all instances tubes that had been clear on the 8th or the 10th day, proved sterile. In each experiment the controls and a number of the tubes in which bacteria had grown up were examined by means of stained smears and subcultures to determine the purity of the growth.

With this method different experiments conducted at different times and with the same stock culture of micro-organism yielded very similar results when similar dilutions were used. In other words, if 0.1 c.c. of a 2% solution in 5 c.c. (=1:2500) proved germicidal for a certain micro-organism, this dilution was found uniformly germicidal through several experiments. But different dilutions compared with each other not infrequently showed discrepant results. For example, 0.1 c.c. of a 4% solution in 5 c.c. gave a dilution of 1:2500; likewise, 0.4 c.c. of a 0.5% solution in 5 c.c. gave a dilution of 1:2500. Yet a micro-organism might be killed in the test tube containing the first-mentioned dilution, and might not be killed in the second tube. We have reason to believe that these discrepancies were at times due to errors in technic, as with closer attention to details more uniform results were observed.

For purposes of illustration, the results of a single experiment, selected out of a large number that were conducted by the test-tube method, are shown in Table 4. In this experiment, emetin hydrochlorid and phenol were used, in stock solution of 1%, and a filtered 24-hour extract-broth culture of B. typhosus in dose of 0.1 c.c. to each tube. The culture medium and diluent were extract broth, reaction neutral. The quantity in each tube was sufficient to make a total of 5 c.c.

TABLE 4

RESULTS OF EXPERIMENT SHOWING BACTERICIDAL ACTION OF EMETIN HYDROCHLORID AND PHENOL AGAINST B. TYPHOSUS WITH THE TEST-TUBE METHOD

Solution	Dose,	Final Dilution	Results Over Period of 10 Days									
			1	2	3	4	5	6	7	8	9	10
Emetin hydrochlorid 1%	0.1	1:5000	—	+	+	+	+	+	+	+	+	+
	0.2	1:2500	—	+	+	+	+	+	+	+	+	+
	0.4	1:1250	—	—	+	+	+	+	+	+	+	+
	0.6	1:833	—	—	—	—	—	+	+	+	+	+
	0.8	1:625	—	—	—	—	—	—	—	—	—	—
	1.0	1:500	—	—	—	—	—	—	—	—	—	—
Phenol 1%	0.1	1:5000	+	+	+	+	+	+	+	+	+	+
	0.2	1:2500	+	+	+	+	+	+	+	+	+	+
	0.4	1:1250	+	+	+	+	+	+	+	+	+	+
	0.6	1:833	—	+	+	+	+	+	+	+	+	+
	0.8	1:625	—	—	—	—	—	—	—	—	—	—
	1.0	1:500	—	—	—	—	—	—	—	—	—	—

In this experiment emetin proved antiseptic in a dilution of 1:5000, all tubes remaining perfectly clear after the first 24 hours' incubation, whereas the control tube showed a good growth; at the end of the

10-day period of observation the dilution of 1 : 625 was sterile. Similar results with emetin and B. typhosus were observed in other experiments.

As noted in the table, phenol also proved germicidal for B. typhosus in dilution of 1 : 625. Phenol proved germicidal quickly if at all; that is, tubes found sterile at the end of 48 hours were likely to remain so over the 10-day period of observation. With emetin, however, as before stated, the germicidal action was much slower, so that it was necessary to observe the results over the 10-day period in order to secure accurate determinations.

The results of experiments with emetin and phenol and the various test micro-organisms are summarized in Table 5. The dilutions given are those which proved germicidal through the 10-day period of observation. As previously stated, the results varied slightly with different stock solutions of the same substance, and for this reason the results observed with each solution are placed in the tables.

TABLE 5

SUMMARY OF THE RESULTS IN THE STUDY OF THE GERMICIDAL ACTIVITY OF EMETIN HYDROCHLORID AND OF PHENOL

Substance Used	Test Micro-organisms	Stock Solutions				
		4%	2%	1%	0.5%	0.25%
Emetin hydrochlorid	B. typhosus.....	1:624	1:625	1:625	None*	None†
	Staphylococcus aureus	1:2500	1:2500	1:5000 to 1:1250	1:2500 to none in 1:1000	None
	Streptococcus salivarius	1:624	1:2500	1:2500	1:2500	1:2500 to none
	B. anthracis.....	At least 1:125 to 1:1250	At least 1:2500	1:5000 to 1:1250	1:5000 to 1:6666	1:2000
	B. subtilis.....	At least 1:1250	At least 1:2500	1:5000 to 1:1250	1:2500	1:2000 to none
Phenol	B. typhosus.....	1:624	1:500	1:625	None	None
	Staphylococcus aureus	1:624	1:416 to none in 1:250	None in 1:500	None	None
	Streptococcus salivarius	1:312	1:416	1:500	None	None
	B. anthracis.....	1:312 to 1:625	1:416	1:500	None	None
	B. subtilis.....	1:312	1:416	1:500	None	None

* No germicidal action in the lowest dilution, 1: 1000.
† No germicidal action in the lowest dilution, 1: 2000.

From a study of Table 5 it is evident that with the technic employed, whereby the substance is left in contact with the test micro-organism over the entire period of observation, emetin proved equal to phenol in germicidal power, and was frequently from 1 to 5 times more effi-

cacious than phenol in this respect. The striking results observed with the spore-bearing B. anthracis and B. subtilis suggest that while emetin is a slowly acting germicide, it is capable of attacking and killing the naked germs in spore-germination.

In other experiments 2, 1, ½, ¼, ⅛, 1/16, to 1/512% solutions of emetin, in amounts of 2 c.c. each, in a series of sterile test tubes, prepared with sterile neutral broth as the solvent and diluent, were inoculated with 0.1 c.c. of a filtered 24-hour broth culture of B. typhosus; at the end of 10 days the tubes containing 2, 1, ½, and ¼% solutions were sterile; all others, including the control, showed a good growth of the bacillus. After the first 24 hours all dilutions from 2 to 1/16% inclusive were sterile, but during the succeeding days the 1/16 and ⅛% solutions permitted the bacillus to grow. In a similar experiment conducted with phenol, ⅛% was the highest dilution proving germicidal, and was therein superior to emetin, the highest germicidal dilution of which was ¼%.

With the Plate Method.—We have studied the germicidal activity of emetin and of phenol (as control) in plates of agar agar, after the following method: Stock solutions of emetin varying from 4 to ¼% were prepared in sterile broth as heretofore. A given dilution being used, 0.1, 0.2, 0.4, 0.6, 0.8, and 1 c.c. were placed in a series of sterile Petri dishes. To each dish and the control was added 0.01 c.c. of a filtered 24-hour extract-broth culture of the test microorganism. A flask of plain neutral agar was melted and cooled to 42 C., and by means of a sterile 10-c.c. volumetric pipet sufficient of this agar was added to each plate to make the total quantity in each, 10 c.c., or as close to this amount as was possible when pipetting agar at this temperature. Each plate was thoroughly mixed and all incubated at 37 C.; counts were made at the end of 24, 48, and 72 hours.

Table 6 shows the results of a single experiment conducted in this manner. Emetin hydrochlorid and phenol were used, in stock dilution of 2%, and a filtered 24-hour extract-broth culture of B. typhosus in dose of 0.01 c.c. to each plate. In the experiment with phenol plain agar agar (1.5%) was used, reaction neutral. The quantity in each plate was sufficient to make a total of 10 c.c.

The great advantage of the plate method over the test-tube method consists in the worker's ability to count colonies in the plates, and thus to detect finer degrees of germicidal activity. In the test-tube method the result is either positive or negative without any intervening ground.

TABLE 6

RESULTS OF EXPERIMENT SHOWING BACTERICIDAL EFFECT OF EMETIN HYDROCHLORID AND OF PHENOL ON B. TYPHOSUS WITH THE PLATE METHOD

Solutions	Dose	Final Dilution	Results of Plate Counting Over a Period of 72 Hours		
			24 Hr.	48 Hr.	72 Hr.
Emetin hydrochlorid 2%	0.1	1:5000	Uncountable*.....	Uncountable.....	Uncountable
	0.2	1:2500	Uncountable.....	Uncountable.....	Uncountable
	0.4	1:1250	32,000.....	Uncountable.....	Uncountable
	0.6	1:832	Sterile.....	Uncountable.....	Uncountable
	0.8	1:624	Sterile.....	Sterile.....	Sterile
	1.0	1:500	Sterile.....	Sterile.....	Sterile
Phenol 2%	0.1	1:5000	Uncountable.....	Uncountable.....	Uncountable
	0.2	1:2500	Uncountable.....	Uncountable.....	Uncountable
	0.4	1:1250	2800.....	5000.....	11000
	0.6	1:832	Sterile.....	Sterile.....	Sterile
	0.8	1:624	Sterile.....	Sterile.....	Sterile
	1.0	1:500	Sterile.....	Sterile.....	Sterile

* Too many colonies to make a count reliable.

The controls were "uncountable" after the first 24 hours' incubation.

As in the test-tube method, finer gradations in germicidal activity may be secured by using 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 c.c. of the stock solution.

In Table 7 are given the results observed with emetin and with phenol with B. typhosus and Staphylococcus aureus after 72 hours with this technic.

TABLE 7

RESULTS OF EXPERIMENT SHOWING BACTERICIDAL ACTIVITY OF EMETIN AND OF PHENOL AGAINST B. TYPHOSUS AND S. AUREUS WITH THE PLATE METHOD

Micro-organism	Solutions	Results After 72 Hours	
		Emetin	Phenol
Bacillus typhosus.....	4%	1:312*	1:624*
	2%	1:624*	1:832*
	1%	Uncountable (1:1000)†	3000 colonies (1:1000)
	0.5%	Uncountable (1:2000)	Uncountable (1:2000)
	0.25%	Uncountable (1:4000)	Uncountable (1:4000)
Staphylococcus aureus.....	4%	Uncountable (1:250)†	1:624*
	2%	Uncountable (1:500)	1:500*
	1%	Uncountable (1:1000)	Uncountable (1:1000)
	0.5%	Uncountable (1:2000)	Uncountable (1:2000)
	0.25%	Uncountable (1:4000)	Uncountable (1:4000)

* Plates sterile in these dilutions.

† The lowest dilution put up with this stock dilution; so many colonies present that an accurate count could not be made.

If Table 7 is compared with Table 5, it will be noted that emetin proved less germicidal with the plate method (or in a solid medium) than with the test-tube method (or in a fluid medium); especially was this true with the culture of S. aureus. It is probable that the drug diffuses slowly, so that in solid media the test micro-organisms escape destruction and multiply to a greater extent than in fluid media. Dr. Robert A. Keilty (personal communication), testing out emetin against

B. coli and an undetermined diplococcus by the method of Cheyne[14] (which is partly a test of diffusibility of germicidal substances) and a 1:200 incorporation of emetin, found emetin practically without germicidal power, these results corresponding to our own as shown in Table 7.

Germicidal Action of Emetin on the Micro-organisms in Pyorrheal Pus.—As the amount of organic matter, particularly pus, in the diluent and menstruum of a test for germicidal activity modifies the results—through absorption of the drug, formation of new and inert compounds, etc.—we have tested the germicidal activity of emetin against the micro-organisms found in pus, using a salt-solution suspension of pus from the mouths of persons suffering with severe forms of pyorrhea alveolaris.

Ten cubic centimeters of normal salt solution were placed in a test tube, warmed to 37 C. and kept at this temperature. This was then inoculated with pus—secured from pus pockets by means of a heavy, sterilized platinum-wire spade—until the suspension was decidedly milky. This suspension was shaken with sterile beads for 10 minutes and used without filtration. Smears showed a variety of micro-organisms, including gram-positive cocci and diplococci, gram-negative diplococci, gram-positive and negative bacilli, and numerous spirochetes and amebae. The emulsion was ready for use within 15 minutes after securing of the pus, was kept constantly at or about 37 C., and in density corresponded approximately to a bacterial vaccine containing 5 billion bacteria to the cubic centimeter.

TABLE 8

RESULTS OF EXPERIMENT SHOWING BACTERICIDAL ACTIVITY OF EMETIN AND OF PHENOL AGAINST MICRO-ORGANISMS IN PUS FROM PYORRHEA ALVEOLARIS

Solutions	Dilution	Results Over a Period of 90 Minutes						Phenol Coefficient
		1	15	30	45	60	90	
Emetin.....	1:50	+	+	+	—	—	—	200:400 = coefficient 2
	1:100	+	+	+	+	+	—	
	1:200	+	+	+	+	+	—	
	1:400	+	+	+	+	+	+	
	1:800	+	+	+	+	+	+	
Phenol.....	1:50	—	—	—	—	—	—	
	1:100	+	—	—	—	—	—	
	1:200	+	+	+	—	—	—	
	1:400	+	+	+	+	+	—	
	1:800	+	+	+	+	+	+	

All controls showed heavy growths in 24 hours.

Five dilutions (4, 2, 1, 0.5, and 0.25% solutions) of emetin were prepared in normal salt solution, and 1 c.c. of each placed in each of 5 sterile test tubes. To each tube was added 1 c.c. of the emulsion of pus and bacteria; the whole was then shaken gently, and kept in a water bath at a constant temperature of 35 to 37 C. The final dilutions of emetin acting on the bacteria were then 2% (1:50); 1% (1:100); ½% (1:200); ¼% (1:400); and ⅛% (1:800) solutions. At the end of 5, 15, 30, 45, 60, and 90 minutes each tube was subcultured into a tube containing 10 c.c. glucose broth by transfering a 4-mm.

[14] Brit. Med. Jour., 1912, 1, p. 1424. Lancet, 1912, 2, p. 1062. Therap. Gaz., 1912, 36, p. 837.

loopful. Controls of the pus and bacterial emulsion were prepared at the beginning and after the completion of the experiments, by subculturing the emulsion in the same manner. A like experiment was conducted with phenol, the same pus and bacterial emulsion being used.

The results as read at the end of 48 hours are shown in Table 8.

With the 90-minute interval of exposure phenol proved twice as strongly germicidal as emetin. It is worthy of particular note that even a 2% solution of emetin required an exposure of 45 minutes to effect sterilization of the bacterial emulsion, whereas phenol killed in 5 minutes in this dilution, and in 15 minutes in a 1% dilution. The 0.5% solution of emetin, which is commonly used in the treatment of pyorrhea alveolaris, required an exposure of 1.5 hours in this experiment (at a temperature of 35 to 47 C.) to effect sterilization of the bacteria in pyorrheal pus. If these experiments may be accepted as a criterion of the germicidal activity of emetin in the diseased tissues, it is apparent that this activity, while present, is relatively slight, and requires that the emetin remain in contact with the tissues over a considerable period of time.

AMEBACIDAL ACTIVITY OF EMETIN HYDROCHLORID WITH REFERENCE TO ENDAMOEBA GINGIVALIS, GROS

The generally accepted specific amebacidal influence of emetin, which had as its experimental basis the studies of Vedder[6] on the influence of ipecac and emetin on amebae in culture, was substantiated later by experiments of Wherry[7] and others, and brilliantly demonstrated by Sir Leonard Rogers[14] and subsequently by a great number of clinicians in application to cases of amebic dysentery. Its value in the treatment of amebic pyorrhea has been shown by Smith and Barrett,[8] Bass and Johns,[2] and others. The impossibility to date of obtaining pathogenic amebae in successful culture has prevented precise experimentation in this as in other lines of interest in relation to these protozoa; and the information we possess concerning the effects of emetin on both Endamoeba histolytica, Schaudinn, and Endamoeba gingivalis, Gros, is derived from clinical observation rather than from laboratory experimentation. If we were permitted to judge purely from clinical results, and to hold as basis for calculation that in an average adult human being there are, at the least, twenty pounds of fluid capable of acting as solvent and diluent of hypodermatically introduced emetin hydrochlorid, and that successful amebacidal influence has at times followed

as low a daily dosage as one-fourth grain of the drug both in dysentery and in pyorrhea (remembering, too, that it is unlikely that all of an injected dose will at once be diffused from the site of introduction, and making allowance for a reasonable but unknown rate of elimination and fixation), it would be well within reason to believe that such occasional successes are attained by dilutions of the remedy ranging well above 1 : 500,000. But in a precise way we know practically nothing of the quantitative relations of the remedy to these parasites, of the time requirements, or even of the exact mode of attack. James[15] has pointed out, from clinico-pathologic studies on the intestinal amebae, that in the course of the administration of emetin cytoplasmic and nuclear degenerative changes are manifested by the amebae obtained from the dejecta; and one of us,[8] in watching the unstained oral amebae in pyorrheal pus under the microscope, observed that when an emetin solution was allowed to flow under the cover into contact the amebae rapidly became quiescent and rounded, their substance assuming a hyaline and relatively opaque appearance, the clear ectoplasm first manifesting the change, which apparently increased at the expense of the endoplasm, until practically the entire parasite, except possibly a few of its contained globules, which became condensed in the central part of the cell, had become opaque and glossy.

This change was at first believed to indicate the actual death of the parasite, and at present the writers believe that when it is well developed it usually does mean that the protozoan is dead; but we have had ocular proof that some of the amebae which show this change, in at least a mediate but readily recognizable degree, may still retain the power of movement and are therefore still living. If it were possible to grow these organisms artificially and after definite exposure to make subcultures of the exposed material in order to determine the destruction of the amebae or their persistent viability, matters would be very different; in the lack of such ability we believed it worth while to proceed on the assumption that such changes usually indicate death, or presage death, and that at least they show an influence by the drug on the parasite.

We therefore prepared solutions of emetin hydrochlorid in proportions of 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, 1/1024, and 1/2048% strength (1 : 200, 1 : 400, etc., up to 1 : 204800) in normal salt solution, and by means of pipets mixed as accurately and as thoroughly as possible equal parts of these with fresh ameba-bearing pus from pyorrheal pockets (all from same patient)

[15] Amer. Jour. Tropical Dis., 1913, 1, p. 431.

on slides, covered them and sealed the cover with wax, and maintained them at 30 C. in an incubator during the period of observation. This mixture of pus and emetin solution rendered the available strength of the remedy in the preparations 1:400, 1:800, 1:1600, etc., to 1:409600. Control preparations made with normal salt solution replacing the emetin-salt solution were similarly made and cared for. In these, movement was apparent, of a sluggish type, as much as 8 hours after the acquirement of the material from the mouth, and the hyaline change in the parasites was not appreciable. Six hours, therefore, was regarded as a safe observation period.

The series reported in Table 9 will serve to indicate the general result.

TABLE 9

INFLUENCE OF DIFFERENT DILUTIONS OF EMETIN ON ENDAMOEBA GINGIVALIS, GROS, AT 2-HOUR INTERVALS

Dosage Rates	Appearance of the Amebae Over a Period of 6 Hours		
	2 Hr.	4 Hr.	6 Hr.
1:400	Motionless Hyaline Rounded	Motionless Hyaline Rounded	Motionless Hyaline Rounded
1:800	Motile Hyaline Pseudopodia	Motionless Hyaline Rounded	Motionless Hyaline Rounded
1:1600	Motionless Hyaline Pseudopodia	Motionless Hyaline Rounded	Motionless Hyaline Rounded
1:3200	Motile Hyaline Pseudopodia	Motile Hyaline Pseudopodia	Motile Hyaline Pseudopodia
1:6400	Motile Hyaline (clear cytoplasm) Pseudopodia	Motile Hyaline Pseudopodia	Motile Hyaline Pseudopodia
1:12800	Motionless Hyaline Pseudopodia	Motionless Hyaline Pseudopodia	Motionless Hyaline Rounded
1:25600	Motile Hyaline Pseudopodia	Motionless Hyaline Pseudopodia	Motionless Hyaline Rounded
1:51200	Motile Clear cytoplasm (hyaline) Pseudopodia	Motionless Hyaline Rounded	Motionless Hyaline Rounded
1:102400	Motile Clear cytoplasm (hyaline) Pseudopodia	Motionless Hyaline Rounded	Motionless Hyaline Rounded
1:204800	Motile Hyaline (clear cytoplasm) Pseudopodia	Motionless Hyaline Rounded	Motionless Hyaline Rounded
1:409600	Motile Hyaline (clear cytoplasm) Pseudopodia	Motionless Hyaline Rounded	Motionless Hyaline Rounded

The discovery of any motile amebae in a slide was noted as if all were motile, altho this really was far from true, as some motionless, hyaline, round examples were found in all specimens. Throughout the entire observation period active motility of certain flagellates (trichomonads mainly) persisted even in the lower dilutions. Movement of associated motile bacteria was lost practically without reference to the emetin proportions before the close of observation. The persistence of motility in dilutions of 1:3200 and 1:6400 is believed to have been due to a failure properly to mix the pus and emetin solution, as both were rather thick samples, and the motility was particularly noted in amebae well embedded in the denser fields. We are unwilling to say at what time and at what dosage death of the amebae takes place; we are willing to assert that at even the highest dilution employed emetin attacks the amebae and brings about visible structural change within 2 hours (for the lower dilutions we know that this may take place within a few minutes); we believe that these changes are at least prelethal. If we are correct in this belief, our results tally fairly with the rough quantitative conclusions which, as we have suggested, may be drawn from clinical sources.

TRYPANOCIDAL ACTIVITY OF EMETIN HYDROCHLORID

While the highly specific influence of emetin has been generally recognized and is sustained by the observations described, it was considered worth while to include here a brief study of the trypanocidal activity of the drug, not only as a matter of interest in its effect on trypanosomes themselves, but as a means of adding in some measure to our knowledge of its general protozoacidal influence. It was thought, too, that, in view of the suspected relation between these flagellates and spirochetes and the known coincidence of chemotherapy, as exemplified by salvarsan, for treponemata, spirochetes, and in some measure for these higher flagellates, some suggestions might be available from such a study as to the possible influence of emetin on the spirochetes of the mouth. This, of course, is far from being of direct value; and since by the method of Noguchi it is possible to obtain cultures of the various mouth spirochetes, we hope later to be in position to present direct, available data on this relation. Experiments of a preliminary character, conducted along lines similar to those in the following trypanosome study, with thick emulsions of pyorrheal pus showing numerous and various spirochetes as well as bacteria, have yielded suggestive results.

The motility of the spirochetes could easily be seen; and, in general, dilutions of emetin of from 1:400 to 1:12,000 seemed to show spirochetacidal influence within an hour, as far as could be judged on the basis of motility alone.

For the study of trypanocidal influence T. lewisi and T. equiperdum were selected and the following technic worked out for conducting these experiments in vitro.

White rats were infected with the respective strains, and used when a drop of blood from the tail showed, on microscopic examination, large numbers of the trypanosomes. Blood was then secured from the tail or from the heart of the animal, and enough placed in a tube of 1% sodium citrate in normal salt solution warmed to 40 C. for each loopful of emulsion, examined in hanging drop with a 1/6 objective and No. 4 eyepiece (Leitz), to show at least 10 trypanosomes in each field. The blood-trypanosome emulsion was kept at a constant temperature of 40 C. by standing the test tube in a beaker of water at this temperature.

Dilutions of emetin varying from 2% (or 1:50) to approximately 1/600% (or 1:51200) were prepared in warm normal salt solution. One loopful of each dilution of emetin was mixed on a warm cover slide with an equal loopful of trypanosome emulsion, and a hanging drop preparation made and sealed with vaselin, as in conducting the microscopic agglutination test with typhoid bacilli. The final dilutions of emetin acting on the trypanosomes then ran from 1% (1:100) to 1/1024% (1:102,400). Each slide and the controls were marked with the time at which the emetin and trypanosomes were mixed. The slides were placed in an incubator at 40 C. and examined on a warm stage with 1/6 objective and No. 4 eyepiece (Leitz).

In all preparations at least 5 trypanosomes could be seen in each field. In the controls the parasites were always vigorous and actively motile; after 2½ hours, however, the motility was much decreased and for this reason each experiment was terminated within this time.

At varying intervals the slides were examined and the effect of the emetin on the trypanosomes studied. The results were definite and easily read within the limits and defects of this technic. First the trypanosomes lost their active to and fro movements, and remained in one position with constant vibratile movement. Later the latter movement became more and more sluggish and finally ceased. The bodies of the parasites at first were elongated, but later became short and swollen, so that various bizarre and peculiar forms could be seen. As soon as careful examination of a slide showed total loss of motility in all trypanosomes, the time was noted; and for the purpose of these experiments the trypanosomes were regarded as having been influenced by the emetin. We realize the defects of this method of study, chiefly in that absolute loss of motility is no sure indication that the trypanosomes are dead and that disintegration will occur. (We have experi-

mented in a different manner by mixing definite volumes of trypanosome suspensions in sterile test tubes with various dilutions of emetin and after a definite interval of time removing the trypanosomes by centrifugation, washing once with normal salt solution to remove traces of emetin, and injecting the trypanosomes re-suspended in warm salt solution into the peritoneal cavities of a series of rats. The results, however, were irregular, a number of controls remaining sterile; the necessarily large amount of handling and the resultant cooling or chilling of the emulsion in centrifugation had killed the trypanosomes.)

The results of these experiments are summarized in Table 10. The time required to bring about total loss of motility varied in different experiments with trypanosomes of the same strain but from different seed rats. The intervals shown in the table represent the shortest and longest intervals of time, respectively, to bring about these results with the various dilutions of emetin.

TABLE 10

TRYPANOCIDAL ACTIVITY OF EMETIN

Dilutions	Minutes of Time Required to Kill All Trypanosomes	
	T. lewisi	T. equiperdum
1:100	8 to 10	17 to 55
1:200	10	13 to 32
1:400	12	11 to 19
1:800	14	21 to 30
1:1600	15 to 18	30 to 41
1:3200	20 to 40	40 to 50
1;6400	25 to 45	60 to 72
1:12800	30 to 70	100 to 130
1:25600	60 to 90	125 to 140
1:51200	70 to 120	150 to 190
1:102400	80 to 140	

These experiments show that emetin possesses trypanocidal activity. With one hour as a safe interval of exposure, since in that space the controls showed no appreciable spontaneous deterioration, a dilution of emetin of 1:25,000 was destructive for T. lewisi and a dilution of 1:6000 for T. equiperdum. The higher resistance of the pathogenic T. equiperdum is probably to be ascribed to a state of higher vitality and vigor in this parasite. In both cases, however, the trypanocidal activity of emetin in vitro appears to be lower than its amebacidal activity and this was even more evident in our experiments in vivo, which are reported in a separate communication.

DISCUSSION

Interest in this study pertains to the rôle of emetin hydrochlorid not only as an amebacide, but likewise as a bactericide in the treatment of pyorrhea alveolaris and amebic dysentery. We were surprised at the high grade of bactericidal action possessed by emetin, altho this activity is not apparent unless the drug remains in contact with bacteria, preferably in a fluid medium, over relatively long intervals of time.

In the treatment of pyorrhea alveolaris by local application of emetin, the drug should not be used in solutions stronger than 0.5% on account of its local irritant effects on the tissues. Tho 0.25% is germicidal in the test tube, this action is apparent only when the solution of emetin is left in contact with the test micro-organism; a 5% solution, on the other hand, fails to kill B. typhosus in 15 minutes. In view of the fact that most of the drug must be ejected from the pus pockets in the gums, by reason of the movements of the jaw, within a short time after the application has been made, it is reasonable to suppose that the quantity of emetin remaining for a sufficient length of time to exert a bactericidal action must be small indeed.

On the other hand, emetin possesses a very high amebacidal action, as determined not only by studies in vitro, but likewise by studies of the material from pyorrhea alveolaris and from amebic dysentery following the hypodermatic injection of the drug. With a dose of 0.016 gm. administered in this manner to a 60 kilo man, the dilution in the body must be at least 1 : 500,000 or 1 : 1,000,000, if one considers that the total blood and body fluids may be placed conservatively at 20 pounds, and does not allow for local fixation of part of the drug at the site of injection—believing that a constant elimination proceeds. In view of the undoubtedly beneficial results following the hypodermatic injection of the drug and its demonstrated amebacidal powers (with which tentatively we relate the structural influence we have noted), it is reasonable to conclude that the drug exerts its curative effects largely by reason of its amebacidal properties, as in these dilutions a bactericidal action in vitro could not be demonstrated and our studies on the bactericidal action of emetin in vivo were largely negative in their results.

Briefly then, we are of the opinion that emetin hydrochlorid exerts some bactericidal action when applied locally in the treatment of pyorrhea alveolaris, but that its bactericidal activity is entirely secondary to its amebacidal action, being of probably even less influence

when emetin is administered hypodermatically. As far as this may, it means, moreover, that this large margin of specific influence of emetin on amebae is confirmatory of the belief that these oral amebic parasites are an important factor in the pathogenesis of pyorrhea. It does not prove that they are the sole agents by any means; and for other reasons the writers are disposed to maintain the attitude expressed by Smith and Barrett, quoted at the opening of this paper, as to a probable association of etiologic factors.

A logical conclusion would seem to be that pyorrhea alveolaris should be treated locally with emetin, with or without coincident hypodermatic injections of the drug; clinical observations bear out the correctness of this conclusion, as in our experience best results are secured with the local use of the drug combined, in cases accompanied by complications, with hypodermatic medication.

CONCLUSIONS

Emetin hydrochlorid possesses bactericidal properties, but prolonged contact with bacteria is required before this action becomes apparent. A 5% solution of emetin failed to kill B. typhosus in 15 minutes, but with a special technic, in which the drug remains in contact with the test micro-organisms, emetin proved about equal to, or even on occasion 5 times more antiseptic and germicidal than corresponding dilutions of pure phenol.

The bactericidal activity of emetin is more apparent in fluid than it is in solid culture media.

In an emulsion of pus and various bacteria from pyorrhea alveolaris a 2% solution of emetin required 45 minutes to effect sterilization, whereas a corresponding dilution of phenol proved germicidal in 5 minutes or less; a 0.5% solution of emetin required 1½ hours, and a corresponding dilution of phenol, 45 minutes, to sterilize the emulsion.

Emetin hydrochlorid possesses trypanocidal properties in vitro, but this action is probably less vigorous than is its amebacidal action.

Emetin is highly amebacidal, producing a marked structural change in Endameba gingivalis when applied in direct contact, even in high dilution.

Emetin hydrochlorid probably exerts some bactericidal action when applied locally in the treatment of pyorrhea alveolaris; but its bactericidal activity must be entirely secondary in importance to its amebacidal action, in view especially of the beneficial results and the dis-

appearance of amebae following the hypodermatic use of the drug in the treatment of pyorrhea alveolaris and amebic dysentery when the drug is highly diluted in the body fluids.

In view, however, of the probable bactericidal value of emetin when applied locally it would appear that the logical treatment of pyorrhea alveolaris should consist primarily in its local application combined with hypodermatic administration, especially in severe infections or in those accompanied by systemic complications.